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28 **Abstract**

29 Excessive gut luminal iron has been shown to contribute to the initiation and
30 progression of colorectal cancer. However, emerging evidence suggests that
31 reduced iron intake and low systemic iron levels are also associated with the
32 pathogenesis of colorectal cancer. This is of significance due to colorectal cancer
33 patients often presenting with iron deficiency. Iron is necessary for appropriate
34 immunological functions; hence, iron deficiency may hinder cancer
35 immunosurveillance and potentially modify the tumour immune microenvironment.
36 Both of which may assist in cancer development. This is supported by studies
37 showing that colorectal cancer patients with iron deficiency have inferior outcomes
38 and reduced response to therapy. Here, we provide an overview of the
39 immunological consequences of iron deficiency and suggest ensuring adequate iron
40 therapy to limit these outcomes.

41

42 **Introduction**

43 Colorectal cancer is the third most common cancer diagnosed globally and is the
44 second leading cause of cancer mortality. Geographical variability exists within the
45 prevalence of colorectal cancer, most often associated with a western lifestyle.
46 Obesity, poor diet and a decline in physical activity are all known contributors to
47 colorectal cancer development.^{1,2} However, an emerging dietary contributor that has
48 been linked to colorectal cancer is iron.

49

50 Iron is a vital micronutrient that is central to many major biological functions, for
51 instance, its role in the transport of oxygen within the iron-containing haemoglobin
52 complex.³ Irons capacity to be utilised in multiple biochemical reactions is through its

53 ability to transition through multiple oxidation states, within the cell the most
54 commonly found being ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms.⁴ In normal physiology
55 iron is necessary for cellular proliferation. For instance, DNA synthesis requires the
56 iron-dependent enzyme ribonucleotide reductase, which catalyses the rate-limiting
57 step of DNA synthesis. Likewise, iron contributes to cell cycle progression, as it
58 forms an essential part of the electron transport chain, providing energy production
59 for cell cycling. As these processes which are necessary for cellular proliferation are
60 dependent on iron, this allows the potential for iron to be utilised in pathological
61 conditions such as cancer. Iron has been implicated in multiple tumour types, with
62 the most notable being colorectal cancer.⁵

63

64 Excessive intestinal iron within the gastrointestinal (GI) tract has been shown to have
65 a role in increasing the risk of developing colorectal cancer.⁶ This is through its utility
66 in cancer cell proliferation, contributing to oxidative stress-induced colonic damage,
67 as well as amplifying oncogenic signalling.^{7, 8} In contrast, evidence to suggest a role
68 for iron deficit in the pathogenesis of colorectal cancer is less well defined. This is of
69 particular focus for investigation, due to iron deficiency being common in colorectal
70 cancer patients. Iron deficiency can result in the clinical manifestation of anaemia
71 within these patients, through limiting haematopoiesis.^{9, 10} As haematopoiesis
72 produces all immune cells, along with iron being required for immune cell function,
73 this leaves the potential for iron deficiency to cause an attenuated immune
74 response.^{11, 12} If this is occurring in colorectal cancer patients this may lead to a
75 reduced immunosurveillance response and altered tumour immune
76 microenvironment, which has the potential to contribute to cancer progression.¹³

77 Hence, this review will address the consequences of iron deficiency on immune
78 function and provide an insight into iron therapy in order to limit these outcomes.

79

80 **Iron Metabolism**

81 Before we can expand upon the role of iron in colorectal cancer, we first need to
82 appreciate how iron is absorbed and regulated within the body. As iron is necessary
83 for homeostasis its concentration is normally tightly controlled within the body.¹⁴ This
84 occurs through being complexed to proteins that facilitate its absorption,
85 transportation, storage and utilisation (Figure 1).⁴ Iron is present within the body by
86 two means, through absorption or recycling.¹⁵ Dietary iron can be consumed in the
87 form of haem iron (Fe^{2+}) and non-haem iron (Fe^{3+}).¹⁶ Initially, dietary iron is absorbed
88 through the apical surface of enterocytes predominantly of the duodenum and upper
89 jejunum, this is facilitated by both divalent metal transporter-1 (DMT-1) and haem
90 carrier protein 1 (HCP-1).^{17, 18} Non-haem iron is reduced by duodenal cytochrome B
91 (Dcytb) to Fe^{2+} before it can be transported by DMT-1 into the enterocyte. Likewise,
92 HCP-1 absorbs haem iron before being internalised into the intestinal enterocytes.
93 Once intracellular the enzyme haem oxygenase-1 (HO-1) releases iron from its
94 haem complex.¹⁹ These mechanisms collectively contribute to the intracellular iron
95 pool; iron then has one of two fates dependent on the body's requirements for iron. If
96 there is no iron requirement within the body, then the iron remains inert bound to the
97 intracellular storage protein ferritin.¹⁸ This may be carried out by the chaperone
98 protein poly binding protein 1 (PCBP1) which facilitates the loading of iron onto
99 ferritin.²⁰ In contrast, if iron is required by the body it will transverse the basolateral
100 surface, by the efflux protein ferroportin (FPN), passing into the circulation.¹⁸ The
101 second mechanism by which iron is released is through the mononuclear phagocytic

102 system (MPS) which regulates iron recycling. MPS is central for iron homeostasis;
103 specialized macrophages recycle iron through engulfment of senescent
104 erythrocytes.¹⁵ These two mechanisms, recycling and absorption, regulate the input
105 of iron into the circulation. Circulating iron is found associated with the plasma
106 protein transferrin in order to maintain it in a redox inert state. Transferrin functions to
107 deliver iron to all tissues, including the bone marrow for erythropoiesis, through
108 binding to the cells surface transferrin receptor.¹⁷

109

110 The absorption of iron is regulated by local iron levels, along with systemic factors.
111 At the local level, iron concentration regulates the iron regulatory protein RNA
112 binding activity, which in turn alters the levels of DMT-1 and FPN.¹⁸ Systemic signals
113 regulating the body's iron requirements are sensed by the liver, which in turn alters
114 the expression of the iron regulatory hormone hepcidin. Hepcidin binds to the FPN
115 leading to internalisation and degradation, which in turn limits the absorption of iron
116 in the small intestine. Similarly, hepcidin regulates iron MPS recycling by causing
117 sequestration of iron into macrophages.²¹ Factors influencing the liver's production of
118 hepcidin include the iron stores, erythropoiesis rate, hypoxia and inflammation
119 (Figure 1). During inflammation, the cytokine IL-6 is necessary for the induction of
120 hepcidin. This is seen during infection when pathogenic macromolecules such as
121 lipopolysaccharides act on macrophages, such as the hepatic Kupffer cells, to
122 induce production of IL-6 which in turn acts on hepatocytes to cause hepcidin mRNA
123 production.²²

124

125 **Excess and Deficit of Iron in Colorectal Cancer**

126 Only 10% of iron consumed in a typical diet is absorbed in the small intestine, this
127 leaves the remnant iron residing within the GI tract.²³ Excessive iron within the GI
128 tract may contribute to increased proliferation and neoplastic conversion of colonic
129 cells. Rodent studies have revealed increased dietary iron enhances proliferation of
130 colonic crypt cells and amplifies colorectal tumour development.^{24, 25} This may be
131 through iron's utility in cellular proliferation, along with its ability to switch between
132 oxidation states, which is tremendously toxic due to the production of reactive
133 oxygen species from the Fenton's reaction.⁴ Reactive oxygen species increase
134 oxidative stress within the GI tract that contributes to DNA damage, modification of
135 proteins and lipid peroxidation. Oxidative stress-induced damage to DNA may lead
136 to genomic instability that can contribute to carcinogenesis.⁷ Similarly, excess iron-
137 induced oxidative stress can contribute to colonic inflammation.²⁶ Chronic
138 inflammation of the colon can contribute to colorectal cancer through the production
139 of growth factors and cytokines that can support tumour growth, disrupt
140 differentiation and promote cancer cell survival.^{27, 28}

141

142 The mechanisms linking excessive iron and colorectal cancer are further supported
143 through the tumour suppressor gene adenomatous polyposis coli (APC). APC is
144 recurrently mutated in sporadic and hereditary colorectal cancer.²⁹ *In vitro* studies
145 have shown that excessive iron in a background of APC mutation leads to an
146 increase in signalling through the major oncogenic signalling pathway, Wnt.⁸
147 Iron/APC-driven colorectal carcinogenesis has also been supported through an APC-
148 deficient murine model, where luminal iron depletion resulted in a reduction in
149 tumour development, whereas, an increase in luminal iron promoted
150 tumorigenesis.³⁰ These studies suggest an association between iron and colorectal

151 cancer, through increasing oxidative stress, inducing carcinogens and amplifying
152 oncogenic signalling.

153

154 The association between high iron and the risk of colorectal cancer has been well
155 established. However, a study by *Bird et al*³¹ assessed 965 men and women aged
156 50-75 to determine iron intake as a risk for development of adenomatous polyps, a
157 precursor lesion to colorectal cancer. They found a U-shaped association between
158 iron intake and colorectal polyps, showing that individuals that consume high iron
159 (>27.3 mg/day) as well as low iron (<11.6 mg/day) had increased risk, compared to
160 those consuming an adequate amount of iron (11.6-13.6 mg/day).³¹ This suggests
161 that a deficit of iron could equally contribute to the pathogenesis of colorectal cancer
162 as high iron does. A similar study by *Cross et al*³² supports this, showing an inverse
163 association between serum iron levels and the risk of colon cancer.³² This may
164 indicate that the level of iron within the blood may be contributing to cancer
165 development when in shortage, in a similar way that excessive iron within the gut
166 lumen contributes to tumour formation. The mechanism supporting iron deficiency
167 and colorectal cancer development is not fully understood. However, it may involve
168 cellular functions requirement for iron, which when deficient may hinder immune cells
169 ability to protect against cancer.¹³

170

171 **Dietary Iron and Colorectal Cancer**

172 Many dietary components, frequently associated with iron, have been shown to
173 contribute to or have protective roles against colorectal cancer. For instance,
174 phytates are anti-nutrients that form complexes with dietary minerals including iron,
175 leading to a reduction in bioavailability.³³ Phytates are inhibitors of iron-induced

176 production of hydroxyl radicals, a potent oxidant that can contribute to cancer
177 development.³⁴ Likewise, vitamin C has been suggested to have a protective role
178 against cancer through regulating iron. Vitamin C chelates iron, leading to greatly
179 enhanced absorption of iron from the diet.^{35, 36} Vitamin C limits free radical damage
180 through the quenching of reactive oxygen species and has been shown to modulate
181 cancer cell survival.^{37, 38} This has been supported in a large population study
182 showing that dietary intake of vitamin C is associated with a lower risk of colorectal
183 cancer.³⁹

184

185 Dietary iron is present in two forms, haem iron from animal sources such as red
186 meat and non-haem iron from seeds, nuts, grains and dark green leafy vegetables.^{40,}
187 ⁴¹ Red meat including beef and lamb is characterised by a high myoglobin content
188 which consists of increased levels of haem iron relative to white meat such as
189 chicken.⁴² Dietary components of meat have been shown to contribute to colorectal
190 cancer, for instance, *N*-nitroso compounds are mutagenic and are potent
191 carcinogens.^{43, 44} Likewise, haem iron has also been shown to contribute to
192 colorectal cancer via the Fenton's reaction. Hydroxyl radicals produced by the
193 Fenton's reaction can alter DNA leading to oxidative base damage.⁴⁵ Furthermore,
194 haem iron has been shown to contribute cancer through inducing colonic
195 hyperproliferation through modulation of the intestinal microbiota and inducing
196 mutations through DNA adducts.^{46, 47} Haem iron also leads to the raised formation of
197 lipid peroxyl radicals, such as malondialdehyde and 4-hydroxynonenal, which are
198 potent carcinogens.³ This provides a strong association between haem iron and
199 colorectal cancer, however, no evidence is available to link a mechanism of non -
200 haem iron and colorectal cancer.

201

202 This is supported in a study by *Luo et al*⁴⁸ which assessed the different forms of iron
203 and their association with colorectal cancer. They determined that haem iron was
204 positively associated with colorectal cancer, whereas, non-haem iron showed no
205 positive association. Supporting the role of excessive dietary haem iron, but not non-
206 haem iron, in the pathogenesis of colorectal cancer. Interestingly, along with
207 excessive dietary haem iron, this study also determined that a lower intake of non-
208 haem iron was also associated with colorectal cancer.⁴⁸ This supports previous
209 studies by *Bird et al*^{β1} and *Cross et al*^{β2} showing that low iron is associated with
210 cancer risk, however, this study expands upon this suggesting a reduction of non-
211 haem iron is responsible.

212

213 **Iron Deficiency Anaemia**

214 Colorectal cancer is commonly associated with the development of iron deficiency,
215 which is prevalent in approximately 60% of colorectal cancer patients. Iron deficiency
216 can then lead to the clinical manifestation of iron deficiency anaemia (IDA).⁹
217 Causative mechanisms of colorectal cancer-associated IDA comprises chronic
218 tumour-induced blood loss, reduced luminal absorption of iron and impairment of iron
219 homeostasis prompted by chronic inflammatory disease.⁴⁹ IDA induced through
220 chronic GI bleeding results in a depletion of iron stores which leads to absolute iron
221 deficiency (AID). Whereas, reduced uptake of iron, along with sequestration to the
222 MPS, causes a decline in biologically available iron resulting in functional iron
223 deficiency (FID).⁵⁰ The clinical relevance of the distinction between AID and FID
224 relates to the administration of iron therapy to treat anaemia. AID requires iron

225 therapy irrespective of actual haemoglobin levels, whereas, in FID iron therapy is
226 only recommended if anaemia induced symptoms occur.⁴⁹

227

228 Haematopoiesis is sensitive to iron deficiency, leading to the manifestation of
229 anaemia in iron-deficient colorectal cancer patients.^{10, 49, 51} Haematopoiesis produces
230 erythrocytes as well as immune cells such as T-cells, macrophages, dendritic cells
231 and natural killer cells. Hence, this suggests that the effects of iron deficiency may
232 not be restricted to the erythroid lineage and may influence the development and
233 function of immune cells.^{11, 51} With iron deficiency being so prevalent within colorectal
234 cancer, this review will address the implication of iron deficiency anaemia on immune
235 function and how this may contribute to the pathogenesis of colorectal cancer.

236

237 **Iron Deficiency, Immune system and Infection**

238 Depletion of biologically available iron within the body due to iron deficiency results
239 in the clinical manifestation of anaemia. However, iron is essential for all cells to
240 function.⁵² Iron is critical in maintaining the immune system through regulating
241 growth and differentiation of immune cells.¹² Additionally, iron is essential for
242 components of peroxide and nitrous oxide generating enzymes required for
243 adequate enzymatic functionality of immune cells.⁵³ Therefore, iron deficiency results
244 in impaired cellular immunity, notably leading to defective T-cell maturation⁵⁴, halting
245 of macrophage differentiation⁵⁵ and impaired natural killer cells activity.⁵⁶ The
246 association between iron deficiency and impairment of immune function has been
247 confirmed, as patients with IDA have increased morbidity from infectious disease.⁵⁷

248

249 **Iron Deficiency and Immunosurveillance**

250 The immune system is essential to prevent infection; however, it is also required to
251 detect and eliminate potentially transformed cells before they manifest into a
252 malignancy. Transformed cells begin to express foreign antigen that can be
253 recognised by the immune system. Immune cells act to survey the body for these
254 cancerous or precancerous cells and eliminate them in a process called
255 immunosurveillance.⁵⁸ Hence, in order for cancer cells to survive they need to evade
256 immune destruction, which is a hallmark of cancer.⁵⁹ Iron plays an essential role in
257 immunosurveillance, through its utilisation in growth and differentiation of immune
258 cells, as well as influencing cell-mediated immune response and cytokines
259 activities.⁶⁰ Therefore, iron deficiency provides the potential for a suppressed
260 immunosurveillance response, which may contribute to tumour immune cell evasion
261 and inadequate tumour cell destruction.⁶¹ How iron deficiency alters the major
262 immune cells and cytokines involved in the immune surveillance response are
263 discussed below.

264

265 Dendritic Cells

266 Dendritic cells are the most potent antigen-presenting cells that bridge the innate and
267 adaptive immune systems and are required for activation of the antitumor T cells.⁶²
268 Iron plays a key role in the differentiation of dendritic cells through supporting the
269 induction of the cyclin-dependent kinase inhibitor p21. Iron deprivation of dendritic
270 cells results in an undifferentiated phenotype, with absent or blunted dendritic
271 processes, that are unable to stimulate T cells. Depletion of iron leads to an increase
272 in the cell surface localisation of transferrin receptors on dendritic cells, suggesting
273 that these cells are attempting to acquire iron. Hence, in IDA there may be a

274 reduction in the activation of antitumor T cell response, through impaired dendritic
275 cell function.⁶³

276 T-Cells

277 T cells are critical to immunosurveillance through their various subtypes. Cytotoxic T
278 cells are major effector cells in the immune response against cancer, through their T
279 cell receptors ability to recognise tumour associated antigens on cancer cells.⁶⁴
280 Cytotoxic T cells then induce apoptosis of tumour cells through perforin and
281 granzyme mediated cell lysis.⁶⁵ Helper T cells act to support this mechanism through
282 aiding dendritic cells in activating cytotoxic T cells, along with producing cytokines
283 such as IL-2 and IFN-gamma to recruit and activate T cells and natural killer cells.⁶⁶
284 However, in IDA these mechanisms are suboptimal.

285

286 Iron deficiency has been shown to cause a reduction of circulating T cells through a
287 limitation of T cell proliferation.⁶⁷ Similarly, iron deficiency also leads to a reduction in
288 T cell motility through inhibiting protein kinase C.¹³ The overlying mechanism that
289 results in these iron deficiency-induced T cell dysfunction may be through increased
290 oxidative stress, due to an increase in oxidant levels along with a decrease in
291 antioxidant enzymatic activity associated with iron deficit. This increase in oxidative
292 stress caused by IDA induces DNA damage in lymphocytes; this was confirmed in a
293 study by *Aslan et al*⁶⁸ which showed that lymphocyte DNA damage was significantly
294 increased in patients with IDA. Lymphocyte DNA damage may result in a defective T
295 cell population, contributing to an impaired immune response.^{68,69} T cells act as the
296 key cells in immunosurveillance, which if repressed create a favourable condition for
297 the development and progression of cancer.¹³

298

299 Natural Killer Cells

300 Natural killer cells are specialised cytotoxic cells that play a pivotal role in
301 immunosurveillance through perforin and granzyme mediated tumour cell
302 destruction.⁷⁰ Stress associated ligands are present on tumour cells, such as HSP70
303 and MICA/B. These stimulate natural killer cells through activating receptors, NKG2D
304 and NKp46, allowing natural killer cells to detect cancer cells. However, IDA can lead
305 to an impairment of natural killer cells antitumor activity through the induction of
306 hypoxia. Hypoxia leads to a downregulation of natural killer cell-activating receptors,
307 NKG2D and NKp46, as well as decreasing the presence of stress associated ligands
308 HSP70 and MICA/B on cancer cells.^{71, 72} Hypoxia also leads to a degradation of
309 natural killer cell-derived granzyme B that is required for the elimination of cancerous
310 cells.⁷³ Hence, IDA patients may have reduced immunosurveillance abilities of
311 natural killer cells, which have the potential to allow evasion of cancer cell
312 destruction by the immune system.¹³

313

314 IL-2

315 IL-2 is an essential cytokine regulating immunosurveillance. IL-2 is produced by
316 lymphocytes and is critical for the proliferation of naïve T cells and their
317 differentiation into antitumoral effector T cells.⁷⁴ Release of IL-2 is also vital in the
318 communication between T cells and natural killer cells.⁷⁵ Furthermore, IL-2 is
319 required to stimulate the growth and activity of natural killer cells, in order for them to
320 exert their antitumor responses. Therefore, IL-2 plays a pivotal role in regulating the
321 immunosurveillance cellular response to cancer. However, *in vitro* studies have
322 assessed the implications of iron deficiency on immune cell cytokine production.
323 Demonstrating a significant reduction in IL-2 production in response to iron deficit.

324 This may lead to a reduced antitumoral immune response in patients with IDA, as IL-
325 2 may be required by the immune system to control cancer.⁷⁴ This has been
326 supported in colorectal cancer mouse models, where increased IL-2 expression was
327 associated with inhibited tumour formation and growth.⁷⁶ Therefore, a reduction in
328 IL-2 production associated with iron deficiency creates a dampened immunological
329 environment that may be more passive to cancer development.

330

331 IFN-gamma

332 IFN-gamma is a cytokine released by T helper cells, which acts to aid the
333 immunosurveillance antitumor response through helping to recruit and activate
334 natural killer cells.^{77, 78} Hence, IFN-gamma acting on natural killer cells acts to limit
335 tumour growth and metastasis.⁷⁹ However, iron deficiency leads to a reduction in
336 IFN-gamma secretion.⁸⁰ IFN-gamma is a pleiotropic cytokine that has anti-
337 proliferative, pro-apoptotic and general antitumor effects. Hence a decrease in IFN-
338 gamma in IDA may lead to a hindering of natural killer cell function and creating a
339 protumorigenic cytokine environment.⁸¹ Supporting evidence implicates a decline in
340 IFN-gamma in the pathogenesis of colorectal cancer, as a decline in IFN-gamma
341 contributes to the proliferation of APC mutant cells through altering EGF and Wnt-
342 mediated signalling.⁸² Further supportive evidence has shown that colorectal cancer
343 patients with lower levels of IFN-gamma, present with significantly worse survival.⁸³
344 This data suggests that IFN-gamma has a protective role against colorectal cancer,
345 which is dampened through iron deficiency.

346 Iron Deficiency and Tumour Microenvironment

347 IDA may alter immune cell function leading to an insufficient immunosurveillance
348 ability of the immune system, which could aid in tumour development. Additionally,

349 iron deficiency may also alter immune cells within the tumour microenvironment,
350 causing them to exert a protumourgenic response.

351 Macrophages

352 Red blood cell haem degradation contributes to iron recycling in the MPS. Spleen
353 and liver macrophages are responsible for this by converting haem to ferrous iron
354 through the expression of high levels of the enzyme HO-1. Tumour-associated
355 macrophages (TAMs) are present in the tumour microenvironment and dependent
356 on their polarization phenotype contribute to tumour development or regression , in
357 part through regulating iron availability for use in cancer cell proliferation. M1
358 classically activated macrophages are pro-inflammatory, express high intracellular
359 levels of ferritin that sequesters iron and promote tumour regression. Whereas, M2
360 macrophages favour tumour growth in part through upregulation of HO-
361 1 mediated iron generation and increased iron export to the tumour
362 microenvironment. HO-1 is expressed in residential macrophages and recruited
363 monocytes within the tumour stroma, hence iron recycling in the tumour
364 microenvironment is dependent heavily on the TAM activity.^{84, 85} *In vitro* studies have
365 shown that depending on TAM polarisation there are different tumour responses,
366 through HO-1-induced iron production and export to the tumour microenvironment.
367 Conditioned media from M2 macrophages lead to more effective stimulation of
368 cancer cell proliferation than that of M1 macrophages. This was dampened by iron
369 chelation , suggesting that increased iron export to the tumour microenvironment by
370 M2 macrophages is responsible for enhanced cellular proliferation.⁸⁶

371

372 Therefore, in individuals with IDA, there may be the potential for there to be a
373 decrease in the iron-sequestering M1 macrophages and an increase in the iron-

374 releasing M2 macrophages. This has been supported *in vitro* were bone marrow-
375 derived macrophages treated with iron showed an increase in M1 polarisation
376 phenotype, while decreasing M2 phenotype.⁸⁷ Hence, in individuals with iron
377 deficiency there may be a reduction in M1 polarisation and a loss of inhibition of M2
378 polarisation. Similarly, research into the effects of iron therapy on immune function
379 against cancer has revealed that iron-loaded TAMs induced through injection of iron
380 oxide nanoparticle lead to reduced tumour size within *in vivo* models. This occurs
381 through the iron treatment leading to repolarization of TAMs to exert an antitumor
382 effect.⁸⁸

383

384 Previously discussed is the role of IFN-gamma deficit induced by iron deficiency
385 driving colorectal cancer. This is further supported in a study using APC deficient
386 multiple intestinal neoplasia mice that lack IFN-gamma signalling. This lack of IFN-
387 gamma signalling led to an accumulation of TAMs which were more prone to M2
388 polarisation.⁸¹

389 Regulatory T-cells

390 Regulatory T-cells (T-Regs) have an indispensable function in peripheral tolerance,
391 preventing detrimental immunopathological responses against self and unharmful
392 foreign antigens.⁸⁹ The risk of developing cancer increases as a result of unregulated
393 immunological responses, as seen in patients with inflammatory bowel disease who
394 have increased risk of developing colorectal cancer. This occurs through multiple
395 mechanisms, for instance, persistent activation of the immune system in chronic
396 inflammation can contribute tumour promoting growth factors and cytokines. Anti-
397 inflammatory CD4+ T-Regs act to reinstate immune homeostasis during chronic
398 inflammation.^{90,91} However, *in vitro* studies have assessed the implications of iron

399 deficiency on T-Regs. Iron chelation resulted in impaired T-Reg activation and
400 proliferation.⁹² This suggests that iron deficiency may lead to the loss of the
401 immunosuppressive effects of T-Regs in the tumour microenvironment, which may
402 contribute to chronic inflammation which is associated with colorectal cancer.

403

404 **Implications of Iron Deficiency on Colorectal Cancer**

405 The consequence of iron deficiency anaemia on the immune system's ability to
406 prevent cancer

407 A 2015 population study conducted by *Hung et al*⁹³ evaluated the risk of cancer in
408 patients with IDA. They determined that there was a significantly increased risk of
409 developing cancer in patients with IDA, irrespective of age or gender.⁹³ A similar
410 study by *Ioannou et al*⁹⁴ assessed explicitly GI malignancies, with their findings
411 revealing GI tumours to be more prevalent in men and postmenopausal women with
412 IDA compared to those with normal iron levels. However, this was not seen in
413 premenopausal women.⁹⁴ These studies suggest an association between IDA and
414 the development of cancer. Which may be due to an impairment of immune function,
415 allowing tumour cell evasion through diminished immunosurveillance or due to a
416 switch to protumorigenic immune cell function within the tumour microenvironment.
417 Further supporting this, in animal studies oral carcinogen lead to earlier cancer
418 development in iron-deficient rats, compared to those with normal iron levels.⁹⁵

419

420 Iron deficiency anaemia exacerbates colorectal cancer

421 Patients with IDA have been shown to have an increased risk of developing tumours.
422 However, IDA is commonly present in patients with preexisting colorectal cancer.
423 Hence, impairment of immune function by iron deficiency may exacerbate the

424 patients' pre-existing cancer. This may be through limiting the immune system's
425 ability to limit tumour growth, hindering responses to therapy and restricting the
426 immune system's response to circulating tumour cells which may develop into distant
427 metastasis.⁹⁶ This has been assessed in clinical studies which showed that
428 colorectal cancer patients with IDA have inferior outcomes than those without IDA,
429 presenting with worse tumour staging and lower disease-free survival.⁹⁷

430

431 Iron deficiency anaemia and response to therapy

432 IDA may also lead to a reduced response to therapies, such as surgery and
433 chemotherapy. Preoperative anaemia in colorectal cancer patients, usually induced
434 through iron deficiency, leads to a decreased survival following surgery.⁹⁸ This may
435 be due to the immune's systems requirements to prevent dissemination of cancer
436 cells following surgery. IDA may impair immune function allowing circulating tumour
437 cells induced through surgery to be undetected and to form metastasis. Similar
438 evidence has supported the fact that anaemia leads to inferior patient outcomes
439 following treatment, a study by *An et al*⁹⁹ showed that patients with preoperative
440 anaemia treated with adjuvant FOLFOX chemotherapy presented with a worse
441 prognosis, than those without anaemia.⁹⁹

442

443 Iron Therapy

444 Oral iron therapy is the current standard treatment for IDA.¹⁰⁰ However, this has
445 been shown to increase the concentration of luminal iron within the GI tract, which
446 increases oxidative stress and inflammation that may contribute to the progression of
447 colorectal cancer.¹⁰¹ In contrast, studies assessing preoperative IDA in colorectal
448 cancer patients have shown that intravenous iron therapy replenishes iron stores

449 and treats iron deficiency more effectively than oral iron, without contributing to gut
450 iron concentration.¹⁰² This is further supported in patients with FID that have reduced
451 luminal iron absorption, therefore oral iron is poorly absorbed in the duodenum of
452 these patients and can contribute to gut iron concentration. Whereas, intravenous
453 iron is more effective at treating iron deficiency in FID patients.^{49, 50}

454

455 Oral iron supplementation has been shown to have a variety of GI side-effects
456 including abdominal pain, dyspepsia, constipation and diarrhoea. Leading to non-
457 compliance as a result of these side-effects. In contrast, intravenous iron has been
458 shown to be better tolerated, with fewer GI side-effects.¹⁰³ Furthermore, many
459 studies have been conducted to support the efficacy and safety of intravenous iron
460 therapy preoperatively in colorectal patients with IDA. A 2020 study by *Kam et al*¹⁰⁴,
461 showed that colorectal cancer patients with IDA who received intravenous iron
462 therapy had significantly increased haemoglobin levels prior to surgery, as well as
463 requiring less red blood cell transfusions, compared to patients not treated with
464 intravenous iron. This study also supported the safety of intravenous iron, stating
465 that they observed no iron-related adverse events following treatment.¹⁰⁴

466

467 This suggests that intravenous iron may provide a more adequate therapy to treat
468 IDA in colorectal cancer patients, compared to oral iron supplementation.

469 Intravenous iron may be a more beneficial therapy by providing optimum repletion of
470 iron stores, in order to ensure normal immune function.

471

472 **Conclusion**

473 Multiple studies have shown that excessive gut luminal iron contributes to colorectal
474 carcinogenesis, through increasing oxidative stress, contributing to inflammation and
475 providing iron for cancer cell proliferation. However, the implication of iron deficiency
476 on colorectal cancer has not been fully assessed. This is of prominent need for
477 investigation due to colorectal cancer patients often presenting with IDA. Iron is
478 necessary for correct immunological function. Hence, iron deficiency may result in a
479 dampened immunosurveillance response, most notably leading to impairments of
480 dendritic cells, T-cells and natural killer cells. Along with this, iron deficiency can
481 modify macrophage polarization and alter T-reg populations, promoting a
482 procarcinogenic tumour immune microenvironment. Collectively these mechanisms
483 may link why patients with IDA have significantly increased risk of developing
484 cancer, have worse colorectal cancer tumour staging, reduced disease-free survival,
485 and reduced response to therapy (Figure 2). Therefore, in order to limits these
486 outcomes, adequate iron therapy is necessary. Oral iron is typically given to
487 colorectal cancer patients with IDA; however, this increases gut luminal iron
488 concentration and does not provide optimum replenishment of iron stores. Studies
489 assessing iron therapy in colorectal cancer patients with IDA have shown that
490 intravenous iron was more effective at replenishing iron stores and treating iron
491 deficiency anaemia than oral iron supplements. This suggests that intravenous iron
492 therapy may be more beneficial in supporting cancer immunosurveillance and
493 limiting pro-tumorigenic microenvironment, without the oncogenic consequences of
494 increasing gut luminal iron.

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505

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766 **Figure 1: Schematic representation of systemic iron regulation.**

767

768 **Figure 2: Summary of how colorectal cancer causes iron deficiency anaemia**
769 **leading to altered immune function that contributes to cancer progression.**